Interface Dynamics of Lipid Membrane Spreading on Solid Surfaces

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As a model system for two-dimensional interface dynamics, we study the wetting front of a lipid membrane moving over a solid substrate that is structured with regularly spaced pinning centers. By analyzing the contour of the front, we derive the normal growth rate and the relaxation coefficient. Both exhibit a $1/t^{1/2}$ time dependence. Moreover, the friction coefficient and the line tension can be determined. Randomly distributed pinning centers cause a fractal contour line, whereas on surfaces that are artificially roughened, self-affine contour lines are observed. The latter exhibit an anomalous roughness exponent of $\zeta = 0.81 \pm 0.05$.

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Phospholipid membranes are a unique example of a two-dimensional fluid. At a water-solid interface they are capable of spreading laterally, if a lipid source provides sufficient material. As a result, a solid surface is covered by a continuous lipid bilayer. Such substrate-supported membranes on planar solids are recently of great interest for applications in biocompatible coatings and sensoric interfaces [1]. In this context, the wetting behavior of lipids on various surfaces was studied and found to be instrumental in preparing supported lipid bilayers [2]. Obviously, substrate heterogeneities will play an important role. Pinning phenomena and contact angle variations are well investigated for spreading of simple liquid droplets on rough or structured surfaces [3]. In particular, a systematic study of the relaxation modes of a periodically deformed liquid interface on a solid was carried out by Ondarcuhu and Veyssie [4]. In this well defined process the experimental dispersion relation of the collective interface modes was determined and found in good agreement with the theoretical prediction by Joanny and de Gennes [5]. On the other hand, if liquid invades a field of random obstacles, the interface line is subject to statistical fluctuations and exhibits randomness. Nevertheless, the morphology and dynamics of liquid driven through disordered media comprises universal properties, such as depinning, dynamic roughening, and self-organized criticality [6,7].

In this Letter we present direct observation of lipid flow on solid surfaces and describe the system as a two-dimensional fluid invasion problem as schematically shown in Fig. 1. We analyze the evolution of the advancing interface line. Using the analytic solution of the deterministic KPZ equation, proposed by Kardar, Parisi, and Zhang [8], the normal growth rate, λ, of the interface height and the relaxation coefficient, ν, are determined for each row of obstacles passed by the membrane. The long-time dynamics of the propagating membrane interface exhibits a slowing down due to the increasing transport length of material from the lipid source. Furthermore, we show that the statistical roughness of the propagating front on surfaces with quenched spatial disorder is in agreement with models for flow in porous media.

In a typical spreading experiment, dry lipid material is stamped from a lipid coated Teflon block onto a clean, hydrophilic glass cover slip. The sample is dried in vacuum for two hours and mounted in a temperature-controlled chamber. To start the experiment, water of 40 °C is added. Subsequently, a single lipid bilayer begins to wet the water-solid interface as schematically depicted in Fig. 1. The spreading is reminiscent of a monomolecular precursor film observed in the flow of liquid crystalline droplets at the air/solid interface [9]. However, two differences characterize the spreading of a lipid membrane: first, it is fully immersed in water, and second, the lipid bilayer exhibits an intrinsically fixed molecular thickness. We use membranes consisting of dimyristoylphosphatidylcholine (DMPC) mixed with 0.02% fluorescently labeled lipid (Texas Red-DHPE). Fluorescent micrographs of the advancing membrane interface were recorded with a digital CCD camera (Micromax, Princeton Instruments). Spatial resolution is limited by the numerical aperture of the objective (NA = 0.75); one pixel corresponds to 0.3 μm. In order to distort the membrane flow we designed substrates with

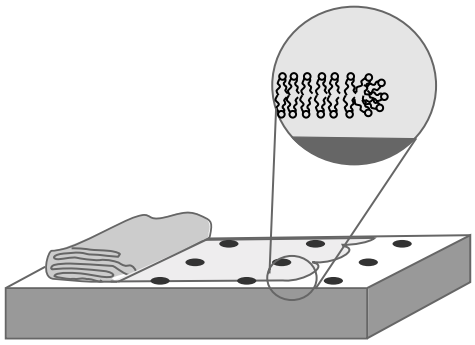

FIG. 1. Sketch of a phospholipid bilayer spreading on glass substrate. The membrane is driven by VdW forces and remains separated from the glass surface by a thin hydration layer.

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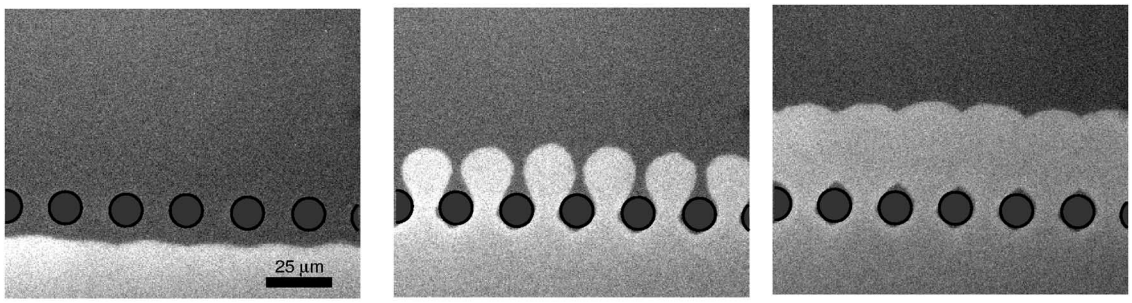

FIG. 2. Time sequence of fluorescently labeled lipid membrane flowing through an array of hydrophobic Al_2O_3 barriers (position indicated by circles). Bar = 25 μm.

arrays of 10 nm high aluminium-oxide barriers. The dots of 7 μm diameter and 25 μm spatial periodicity were made by a standard photolithographical lift-off method. After removal of the photoresist, the substrate was cleaned by an acetone rinse and subsequent UV illumination.

A sequence of fluorescence images illustrates the flow through a line of dots, which are not wetted by the lipid membrane (Fig. 2). The membrane fills the interstitials and spreads from the openings between the obstacles. Circular fingers appear behind the array and grow until they touch. At this time, the spreading front coalesces into a continuous, but undulated interface line, which evolves into a straight front. Two characteristics of the experiment are immediately evident: (i) growth of the rim normal to the interface as manifested in the elementary flow circles and (ii) relaxation of height fluctuations as seen in the smoothing of the distorted interface.

The simplest growth model, which includes both characteristics, is described by the well known KPZ equation. The membrane interface height, $h(x,t)$, follows:

$$\frac{\partial h(x,t)}{\partial t} = \nu\nabla^2 h + \frac{\lambda}{2}(\nabla h)^2 + \eta(x,t). \qquad (1)$$

The first term on the right-hand side describes the interface relaxation. The second, nonlinear term is the leading term in the gradient expansion of a constant normal growth rate [6]. Finally, $\eta(x,t)$ represents noise on the growing interface. We analyze the interface relaxation in the absence of noise ($\eta = 0$). Experimental interfaces were digitized tracing the contour of the lipid flow field using a contour analyzing program. The traces were interpolated by cubic splines to obtain height profiles of equidistant x values. Typical contours of the interface are shown in Fig. 3a.

The first continuous interface after coalescence of the fingers can be used as an initial contour, $h_0(x)$. The evolution of the interface is then calculated using the analytical solution of the deterministic KPZ equation

$$h(x,t) = \lambda t + \frac{2\nu}{\lambda}\ln\int_{-\infty}^{\infty}\frac{d\xi}{\sqrt{4\pi\nu t}} \times \exp\left[-\frac{x-\xi}{4\nu t} + \frac{\lambda}{2\nu}h_0(\xi)\right]. \qquad (2)$$

Note that the variable translation $h \mapsto h + \lambda t$ was used to follow the advancing average height, since h shall be the distance of the advancing front from the lipid reservoir, such that by definition $h(t = 0) = 0$. Figure 3b compares the analytic fit and the experimental contours. An average

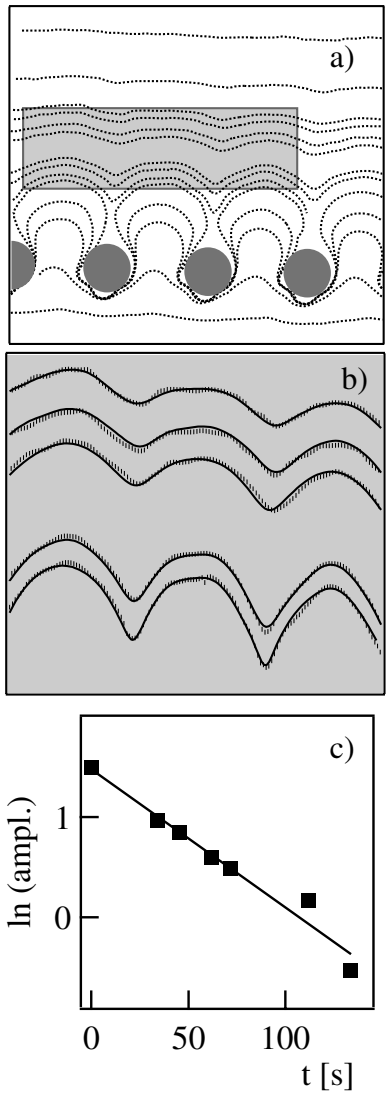

FIG. 3. (a) Image processed traces of the line interfaces followed over a period of 6 min. (b) Blowup of the grey shaded area. The simulated curves (—) are overlaid to experimental data (|). (c) Exponential decay of the first harmonic of the Fourier-transformed interface line.

over five experimental contours yields a best fit for the relaxation coefficient. To check the robustness of the numerical solution we also used an Euler algorithm that directly solves the KPZ differential equation. Stable solutions in agreement with Eq. (2) were found. The relaxation time of the distorted interface is obtained from Fourier analysis of the contours. The first harmonic of the traces depicted in Fig. 3a decays exponentially with relaxation time of about 70 sec.

The parameters λ and ν are assumed constant over the relaxation period, but, in fact, decrease as the membrane passes from one row of obstacles to another. In the following, we determine the parameters λ and ν for successive relaxation events in order to investigate their time dependence. Figure 4 shows a $t^{-1/2}$ decay for λ and ν. This is due to the fact that friction increases with distance, $h(t)$, from the lipid source. Assuming a linear dependence of friction with the source rim distance $f_{\text{visc}} = \gamma_s h(t)\, dh/dt$, the general Washburn's equation $\dot{h} \propto 1/h(t)$ is obtained [10]. Here γ_s denotes the drag coefficient per unit area, which arises from different friction mechanisms as discussed in Ref. [2]. This finding indicates that a complete description of invasion of a regular array has to take into account the transport of material. This needs the nonlocal constraint of mass conservation [10]. In the following we consider the simplest case of a straight interface. Assuming a constant free energy gain per area, S, which is dissipated in the course of sliding, we calculate the average interface height as a function of time

$$h(t) = \sqrt{\frac{2S}{\gamma_s}}\, t^{1/2} \tag{3}$$

and hence the normal growth rate

$$\lambda(t) = \sqrt{\frac{S}{2\gamma_s}}\, t^{-1/2} \tag{4}$$

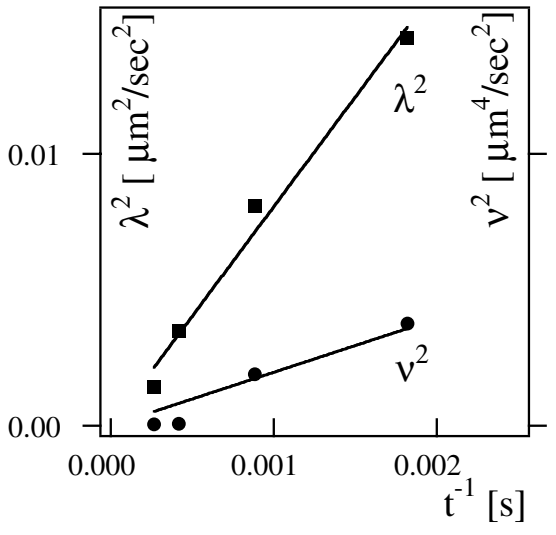

FIG. 4. Slowing down of the growth rate λ (t) and the relaxation parameter ν (t) while passing several rows of obstacles.

with a power law time dependence as measured. Note that the slowing down of the growth rate $\lambda(t) \propto t^{-1/2}$ is not caused by the array of obstacles, but rather by the increasing frictional coupling to the substrate. The same dependence was previously found for freely spreading lipid membranes [2].

The relaxation coefficient, ν, is related to an effective line tension, τ. An elastic restoring force $f_{\text{elastic}} = \tau \partial^2 h/\partial^2 x$ per unit interface length arises, which is balanced by local friction. Using the frictional force as above we yield

$$\tau \approx \gamma_s h(t) \nu \tag{5}$$

and consequently

$$\nu(t) = \tau \sqrt{\frac{1}{2S\gamma_s}}\, t^{-1/2}. \tag{6}$$

The data shown in Fig. 4 allow one to estimate the line tension and the friction coefficient. The spreading power, S, of neutral membranes is dominated by van der Waals (VdW) interactions, and hence $S \approx 2 \times 10^{-4}$ J/m^2. Hence, we obtain a dragging coefficient $\gamma_s = 2 \times 10^6$ N s/m^3 using Eq. (3) and a line tension $\tau = 2.5 \times 10^{-12}$ N $= 0.06kT$ Å^{-1} from Eq. (5). Similar values for line tension have been reported previously from experiments involving pore formation of vesicles [11].

Our experiment on lipid invasion of a regularly structured surface can be extended to the study of flow in quenched spatial disorder, if we are able to create surfaces with a random distribution of defects. Two strategies were followed. First, thin polystyrene films with holes are used as masks for vapor deposition of microscopic Al_2O_3 obstacles on glass. As shown previously by Jacobs *et al.* [12] spatially uncorrelated holes are formed during the early dewetting stage of polystyrene films on silanized SiO_2 surfaces. This procedure allows us to prepare substrates with obstacles as studied above, but with smaller and randomly distributed pinning sites. Second, glass surfaces were treated by vapor deposition of 5 Å silicon monoxide, in order to yield rough, but chemically homogeneous surfaces. Figure 5 depicts different interface morphologies obtained for a spreading lipid. On rough glass surfaces compact lipid flow with rough but self-affine boundary lines is observed. In contrast, membranes flowing across a surface with randomly distributed Al_2O_3 dots lead to fractal-like interface morphologies (Figs. 5b and 5c).

In case of the self-affine interfaces (Fig. 5a) the interface line was digitized and the height-height correlation function

$$G(x,t) = \langle |h(\xi + x, t) - h(\xi, t)|^2 \rangle_\xi \tag{7}$$

determined. Figure 5d shows a log-log representation of $G(x)$ for different times. The initial slope yields scaling of $G(x, t_0) \propto x^{2\zeta}$ with time independent roughness exponent, $\zeta = 0.81 \pm 0.05$.

These results are in good agreement with the two-dimensional model for fluid invasion in porous media

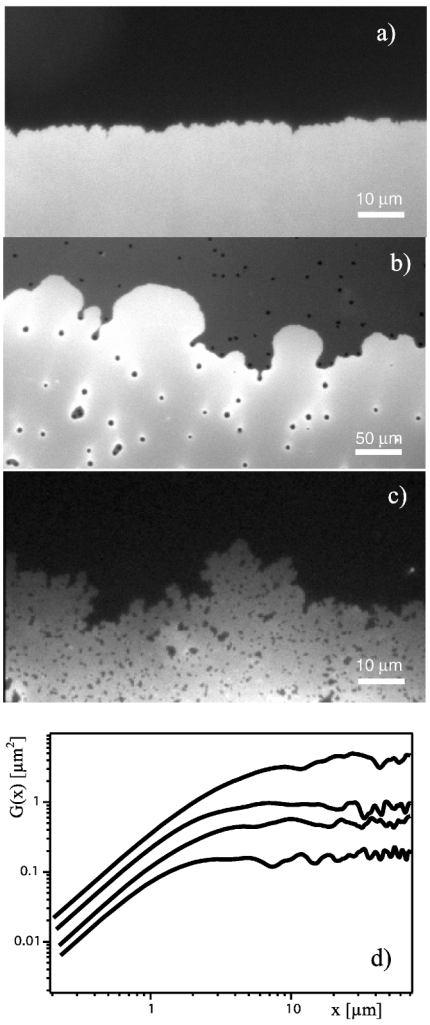

FIG. 5. Fluorescence micrographs of spreading fronts on surfaces with random distributions of defects. (a) Glass surface roughened by vapor deposition. (b) Glass surface with a sparse distribution of randomly distributed Al_2O_3 dots. (defect density $c = 10^{-1}\ \mu\mathrm{m}^{-2}$). (c) Surface as shown in (b) but with defect density $c = 10^{-3}\ \mu\mathrm{m}^{-2}$. (d) Height-height correlation functions of the self-affine contour shown in (a).

proposed by Martys *et al.* [13]. Their theory predicts that fluid invasion of porous media with wetting obstacles exhibits a transition from percolation to depinning, if the contact angle $\theta < \theta_c = 49°$. This approach yields the same roughness exponent $\zeta = 0.81$ for self-affine interfaces in the depinning regime. Similar anomalous roughness exponents were also found in paper imbibition experiments [14].

We analyzed the spreading of lipid membranes on a structured solid surfaces. The interface evolution around defined obstacles is well described by the deterministic KPZ equation on short time scales. On longer time scales the KPZ theory does not account for the slowing down of the membrane spreading rate as well as relaxation coefficient. The observed $t^{-1/2}$ dependence is typical for nonlocal interface dynamics, where the transport of material to the front must be taken into account [10]. However, the phase-field model of imbibition of Dube *et al.,* which includes liquid conservation, does not yield the observed roughness exponent for membrane spreading on rough surfaces. Here the fluid invasion model by Martys *et al.* [13] seems to describe the observed anomalous kinetic roughening best. In particular, our experiments confirm the transition from self-similar to self-affine interfaces as a function of wettability of the pinning obstacles. In conclusion, the spreading of lipid membranes presents a novel experimental realization of $(1 + 1)$-dimensional growth.

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